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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/803,667	03/18/2004	Yasuhiro Sakai	3029-74DIV	6011

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EXAMINER	
HA, JULIE	

ART UNIT	PAPER NUMBER
1654	

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04/25/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/803,667	SAKAI ET AL.	
	Examiner	Art Unit	
	JULIE HA	1654	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *14 February 2008*.

2a)☒ This action is **FINAL**. 2b)☐ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *20,21 and 24-31* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *20,21 and 24-31* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☒ Certified copies of the priority documents have been received in Application No. 10/005753.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Amendment after Non-final rejection filed on February 14, 2008 is acknowledged. Claims 22-23 and 32-34 have been cancelled. Claims 20-21 and 24-31 are pending in this application. Claims 20-21 and 24-31 are examined on the merits in this office action.

Withdrawn Rejections

1. Rejection of claims 20, 25-28 and 30-31 under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement is hereby withdrawn due to Applicant's amendment to claim 20.

Maintained Rejection

Rejection-35 U.S.C. § 112, 1st

2. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

3. Claims 20-21 and 24-31 are rejected are under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The factors to be considered in determining whether a disclosure meets the enablement requirement of 35 U.S.C. 112, first paragraph, have been described in *In re Wands*, 8 USPQ2d 1400 (Fed. Cir. 1988). Among these factors are: (1) the nature or the invention; (2) the state of the prior art; (3) the relative skill of those in the art; (4) the predictability or unpredictability of the art; (5) the breadth of the claims; (6) the amount of direction or guidance presented; (7) the presence or absence of working examples; and (8) the quantity of experimentation necessary. When the above factors are weighed, it is the examiner's position that one skilled in the art could not practice the invention without undue experimentation.

(1) The nature of the invention:

The invention is drawn to a method of staining, and detecting and counting bacteria in clinical samples, in specifically, bacteria in urine samples, and a diluent for bacterial stain. Furthermore, the invention is drawn to staining and detecting bacteria even if a sample contains nitrite ions at high concentrations.

(2) The state of the prior art:

The use of dyes, such as fluorescent, cyanine, methine or polymethine, for staining viable cells, including prokaryotic cells such as bacteria, nucleated eukaryotic cells such as white blood cells, various tumor cells, and mammalian cells in culture (see US Patent # 4783401, column 4, lines 10-14, cited in the previous office action).

Inoue J (US Patent # 5891733) discloses a reagent for analyzing solid components in urine and a method for analyzing solid components in urine the reagent, and more particularly to a reagent employed for an optical analysis of solid components in urine by applying flow cytometry and a method for analyzing the sample (see column 1, lines 9-14). The reference further discloses that examples of solid components include erythrocytes, leukocytes, epithelial cells, urinary casts, bacteria, fungi, crystals and mucus threads. Analyzing these components in urine is of great importance for early discovery of renal and urinary diseases (see column 1, lines 19-23). The reference discloses dyes capable of being excited by a red wavelength light as the first dye to stain the solid components of urine. The reference discloses NK-2782 that is the same dye claimed in instant application as dye (2) (see column 7, lines 1-6 and 35-40).

Akai et al (US Patent # 5891731)discloses a reagent for measuring reticulocytes and also a method of measuring them (see column 1, lines 8-10). The reference further discloses that reticulocytes are young erythrocytes immediately after a release of denucleated erythroblastic cells in bone marrow into peripheral blood (see column 1, lines 15-17). Furthermore, the reference discloses a reagent for measuring reticulocytes comprising at least one dye, which specifically stains reticulocytes, and at least one dye which specifically stains the leukocytes (see column 2, lines 50-53). The reference discloses a compound represented by formula (I) that is the same compound as the compound claimed in the instant application as dye (10) (see column 3, lines 45-55). The reference discloses that this compound represented by formula (I) can specifically stain reticulocytes (see column 3, lines 39-45). The reference further discloses a

cationic surfactant represented by formula (IV) as a staining promoter (see column 8, lines 65-67). The surfactant represented by formula (IV) is the same surfactant claimed in the instant application (see column 9, lines 1-5). Furthermore, the reference discloses that the cationic surfactant has the action of sphering the erythrocyte cells and, therefore, it can converge the intensity distribution of the forward scattered light of the population of the erythrocytes. As a result thereof, discrimination between thrombocytes and erythrocytic cells becomes easy (see column 9, lines 32-37).

Yue ST (US Patent # 5656449) discloses the preparation and use of fluorescent stains for nucleic acids derived from neutral unsymmetrical cyanine dyes comprising a substituted benzazolium ring system linked to a methine bridge to a pyridine or quinoline ring system. The reference further discloses that the dyes have particular utility in the staining of reticulocytes (see abstract). The reference further discloses that the dyes have greater stability in buffered solutions than in water alone; and agents that reduce the levels of oxygen radicals, such as β-mercaptoethanol, contribute to the stability of the dyes (see column 6, lines 18-21). Furthermore, the reference discloses that the cell types for which the dye is an effective nucleic acid stain include cells with or without nuclei, including eukaryotes, including pollen and gamete cells; prokaryotes, particularly bacteria, including both Gram-negative and Gram-positive bacteria; as well as yeast and other fungi, and spores (see column 8, lines 9-165).

The art provide guidance as how the dyes can stain cell types that include eukaryotes, including pollen and gamete cells; prokaryotes, particularly bacteria, including both Gram-negative and Gram-positive bacteria; as well as yeast and other

fungi, and spores. Akai et al and Yue ST disclose dyes that can distinguish and differentiate blood cells. For example, Yue discloses that the dyes of the invention are used to differentiate reticulocytes from other components of a blood sample (see column 8, lines 20-24). Akai et al also discloses cationic surfactants are used as staining promoters. Yue ST discloses that β-mercaptoethanol contribute to stabilizing the dyes. However, none of the prior arts provide guidance as how to distinguish bacteria from urine, when solid components of urine contains erythrocytes, leukocytes, epithelial cells, urinary casts, bacteria, fungi, crystals, and mucus threads (see Inoue patent '733, column 1).

(3) The relative skill of those in the art:

The relative skill of those in the art is high.

(4) The predictability or unpredictability of the art:

Applicant's activity is based on the ability to discriminate bacteria in urine from nitrite ions in urine. Since urine contains other solid components such as erythrocytes, leukocytes, epithelial cells, urinary casts, bacteria, fungi, crystals, and mucus threads, the predictability in the art is low. This is due to the fact that the art has recognized that different blood cells can be differentiated within components of a blood sample, but not when there are other components that are stained by the same dyes. For example, the Yue patent discloses that the cell types that can be stained by the dyes are

erythrocytes, leukocytes, epithelial cells, urinary casts, bacteria, fungi, crystals, and mucus threads.

The claims claim mixing the urine sample and a first reagent comprising a cationic surfactant and a substance capable of reducing nitrite ions. However, as disclosed by Yue and Akai et al patents, a substance capable of reducing nitrite ions (b-mercaptoethanol) stabilizes the dye in solution, and cationic surfactant is staining promoter. The Applicant has not shown how bacteria can be discriminated from other solid components such as erythrocytes in the urine that is stained by the dye. There are too many variables within the samples, thus, it clearly shows the unpredictability of the art.

(5) The breadth of the claims:

The claims are drawn to a method for discriminating bacteria contained in urine sample, comprising: mixing the urine sample and a first reagent comprising a cationic surfactant and a substance capable of reducing nitrite ions; mixing obtained mixture and a second reagent comprising a polymethine dye for staining bacteria; introducing the assay sample into a detecting part of a flow cytometer; and discriminating the bacteria from other components based on the measured scattered light and fluorescent light.

(6) The amount of direction or guidance presented and (7) The presence or absence of working examples:

Although the specification provides guidance on how to discriminate bacteria from other components, such as contaminants (mucus, crystals, amorphous salts and cell fragments that are clinically insignificant) by measuring the intensity of scattered light signal and an intensity of fluorescent light signal or a pulse width reflecting the length of particles to count the number of the bacteria (see paragraphs [0007] and [0020]), it is unclear how to discriminate from other components such as erythrocytes and reticulocytes that are also components of the urine that have high affinity for the dyes. The specification discloses that the substance capable of reducing nitrite ions is used to prevent decomposition of the dye caused by the nitrite ions and as a results, dye transmissivity of bacteria is enhanced (see paragraph [0028]). The specification further discloses that in order to satin bacteria effectively, the cell membrane of the bacteria may be damaged so that a dye enters cells easily, and a cationic surfactant, an nonionic surfactant or the like may be used for achieving this purpose (see paragraph [0029]). The working example is limited to urine sample containing a large amount of nitrite ions, dye A and ascorbic acid in citric acid-NaOH buffer solution. The staining was carried out and the scattered light and fluorescent light were measured in flow cytometer. The specification discloses that as a control, measurement was performed using a reagent containing no ascorbic acid (see paragraph [0075] and Example 1). Example 1 and Figure 1 discloses that in the case where the reagent without ascorbic acid was used, bacteria were not stained and the fluorescent light intensity was zero. In

contrast, bacteria were stained and detected when ascorbic acid was added (see paragraph [0076]). Example 2 discloses measurement was performed in the same manner as Example 1, but sulfamic acid was used in the diluent. Figure 3 indicates that bacteria were stained and detected as in Example 1 (see paragraph [0077]). Both ascorbic acid and sulfamic acid are substance capable of reducing nitrite ions. The working example however, does not provide guidance as how to distinguish bacteria from other cell types such as erythrocytes found in urine. There are not enough working examples for guidance. For example, as explained above, solid components such as erythrocytes, leukocytes, epithelial cells, urinary casts, bacteria and yeast-like fungi are found in urine samples (see Inoue patent '733, column 3, lines 54-57). Akai et al disclose that cationic surfactants act as staining promoter and that the cationic surfactant has the action of sphering the erythrocyte cells and, therefore, it can converge the intensity distribution of the forward scatted light of the population of the erythrocytes. Yue discloses that the agent such as β-mercaptoethanol contribute to the stability of the dyes.

The specification has not provided guidance in the way of a disclosure to how discriminate bacteria from other solid components such as erythrocytes in urine samples. The specification discloses that the substance capable of reducing nitrite ions and/or the cationic surfactant are added, and thus, dye transmissivity to the bacteria cells is enhanced even if nitrate-reducing bacteria produce nitrite ions in the sample, so that bacteria can be quickly detected with high accuracy (see paragraph [0078]). Furthermore, the specification discloses that since the staining can be easily performed

by merely mixing the sample and the reagent, skill required in Gram staining is eliminated, and the staining step can be easily carried out (see paragraph [0079]). Further, the specification discloses that bacteria whose growth is difficult on medium can also be counted reliably (see paragraph [0081]). The specification does not provide any guidance as to how to distinguish bacteria from other solid components such as erythrocytes. There are no examples provided comparing the bacteria stain intensity to other solid components, such as erythrocytes, in urine sample.

Inoue J (US Patent # 5891733) discloses a reagent for analyzing solid components in urine and a method for analyzing solid components in urine the reagent, and more particularly to a reagent employed for an optical analysis of solid components in urine by applying flow cytometry and a method for analyzing the sample (see column 1, lines 9-14). The reference further discloses that examples of solid components include erythrocytes, leukocytes, epithelial cells, urinary casts, bacteria, fungi, crystals and mucus threads. Analyzing these components in urine is of great importance for early discovery of renal and urinary diseases (see column 1, lines 19-23). The reference discloses dyes capable of being excited by a red wavelength light as the first dye to stain the solid components of urine. The reference discloses NK-2782 that is the same dye claimed in instant application as dye (2) (see column 7, lines 1-6 and 35-40).

Akai et al (US Patent # 5891731)discloses a reagent for measuring reticulocytes and also a method of measuring them (see column 1, lines 8-10). The reference further discloses that reticulocytes are young erythrocytes immediately after a release of denucleated erythroblastic cells in bone marrow into peripheral blood (see column 1,

lines 15-17). Furthermore, the reference discloses a reagent for measuring reticulocytes comprising at least one dye which specifically stains reticulocytes and at least one dye which specifically stains the leukocytes (see column 2, lines 50-53). The reference discloses a compound represented by formula (I) that is the same compound as the compound claimed in the instant application as dye (10) (see column 3, lines 45-55). The reference discloses that this compound represented by formula (I) can specifically stain reticulocytes (see column 3, lines 39-45). The reference further discloses a cationic surfactant represented by formula (IV) as a staining promoter (see column 8, lines 65-67). The surfactant represented by formula (IV) is the same surfactant claimed in the instant application (see column 9, lines 1-5). Furthermore, the reference discloses that the cationic surfactant has the action of sphering the erythrocyte cells and, therefore, it can converge the intensity distribution of the forward scattered light of the population of the erythrocytes. As a result thereof, discrimination between thrombocytes and erythrocytic cells becomes easy (see column 9, lines 32-37).

Yue ST (US Patent # 5656449) discloses the preparation and use of fluorescent stains for nucleic acids derived from neutral unsymmetrical cyanine dyes comprising a substituted benzazolium ring system linked to a methine bridge to a pyridine or quinoline ring system. The reference further discloses that the dyes have particular utility in the staining of reticulocytes (see abstract). The reference further discloses that the dyes have greater stability in buffered solutions than in water alone; and agents that reduce the levels of oxygen radicals, such as β-mercaptoethanol, contribute to the stability of the dyes (see column 6, lines 18-21). Furthermore, the reference discloses

that the cell types for which the dye is an effective nucleic acid stain include cells with or without nuclei, including eukaryotes, including pollen and gamete cells; prokaryotes, particularly bacteria, including both Gram-negative and Gram-positive bacteria; as well as yeast and other fungi, and spores (see column 8, lines 9-165).

There is no clear guidance as to how to discriminate bacteria from other solid components such as erythrocyte in urine sample. Since β-mercaptoethanol (nitrite reducing agent claimed) stabilizes the dye in buffer and cationic surfactant acts as staining promoter and enhanced erythrocyte intensity, more guidance is needed as how to discriminate bacteria from other solid components in urine samples. Since the prior art provide guidance as how to distinguish different blood cells (such as between reticulocytes (erythrocyte) from leukocytes) but not within other solid components such as bacteria and erythrocyte in urine, more guidance is necessary.

(8) The quantity of experimentation necessary:

Since it is uncertain to discriminate the bacteria from other solid components such as erythrocytes in urine samples, and the Applicant have not provided how to distinguish between bacteria and other solid components such as erythrocytes in urine samples, one of ordinary skill in the art would be burdened with undue "painstaking experimentation study" to determine if the dyes specifically bind to bacteria, and if bacteria can be discriminated from other solid components in the urine sample.

Response to Applicant's Arguments

4. Applicant argues that "claim 20 has been amended and all claims are dependent on claim 20. Accordingly, all of the claims as amended are of a scope commensurate with the teaching of the present invention. Such teaching would enable one of ordinary skill in the art to practice the presently claimed invention."

5. Applicant's arguments have been fully considered but have not been found persuasive because the amended claim still does not enable a person of ordinary skill how to discriminate the bacteria from other solid components such as erythrocytes in urine samples, and Applicant has not provided how to distinguish between bacteria and other solid components in the urine sample. Claim 20 now recites, "A method of preparing an assay sample for discriminating bacteria by a flow cytometer, comprising...a second reagent comprising a polymethine dye for staining bacteria; and preparing the assay sample by mixing urine sample, the first reagent and the second reagent..." However, this amendment does not clearly indicate how a bacteria from other solid components found in urine will only be stained by polymethine dye. As described in the rejection above, a substance capable of reducing nitrite ions (β-mercaptoethanol) stabilizes the dye in solution, and cationic surfactant is staining promoter. Applicant has not shown how bacteria can be discriminated from other solid components such as erythrocytes in the urine that is stained by the dye. Akai et al disclose that cationic surfactants act as staining promoter and that the cationic surfactant has the action of sphering the erythrocyte cells and, therefore, it can converge the intensity distribution of the forward scattered light of the population of the

erythrocytes. There are too many variables within the sample, thus, it clearly shows the unpredictability of the art.

Conclusion

6. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a). No claims are allowed.

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to JULIE HA whose telephone number is (571)272-5982. The examiner can normally be reached on Mon-Thurs, 5:30 AM to 4:00 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Cecilia Tsang can be reached on 571-272-0562. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Julie Ha/
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/Anish Gupta/
Primary Examiner, Art Unit 1654